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ENVIRONMENTAL QUALITY RESEARCH
Use of Unicellular Algae for Evaluation of Potential Aquatic Contaminants

Second Annual Report

JAN SCHERFIG
PETER S. DIXON
CAROL A. JUSTICE
RICHARD A. APPLEMAN

WATER RESOURCES LABORATORY
SCHOOL OF ENGINEERING
UNIVERSITY OF CALIFORNIA, IRVINE 92717

NOVEMBER 1977

AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

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AMRL-TR-77-53

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Anthony A. Thomas

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Aerospace Medical Research Laboratory

AIR FORCE/56780/19 December 1977 — 150

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: AMRL-TR-77-53	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE *(and Subtitle)*: ENVIRONMENTAL QUALITY RESEARCH Use of Unicellular Algae for Evaluation of Potential Aquatic Contaminants	5. TYPE OF REPORT & PERIOD COVERED: Second Annual Report 1 June 1976-31 May 1977
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Jan Scherfig, Richard A. Appleman, Peter S. Dixon, Carol A. Justice	8. CONTRACT OR GRANT NUMBER(s): F33615-76-C-5005
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Regents of the University of California, University of California, Irvine, California 92717	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 62202F, 6302-04-17
11. CONTROLLING OFFICE NAME AND ADDRESS: Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson AFB, Ohio 45433	12. REPORT DATE: November 1977 13. NUMBER OF PAGES: 25
14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*	15. SECURITY CLASS. *(of this report)*: UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

propellants
rocket fuels
hydrazine
algal bioassays
safe concentrations
effective concentrations
Dunaliella tertiolecta
Selenastrum capricornutum
symmetrical dimethylhydrazine
monomethylhydrazine
unsymmetrical dimethylhydrazine

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Selenastrum capricornutum and Dunaliella tertiolecta were used as test algae in bioassays to determine the toxic and/or biostimulating effects of hydrazine and methylated hydrazines in various fresh water and marine aquatic environments. Standard batch algal assay procedures were used to determine safe concentrations and effective concentrations for the compounds studied. Chemical analyses were performed to determine compound stability under the various test conditions.

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PREFACE

This is the Second Annual Report of work performed under the Air Force Contract AF33615-76-C-5005 and covers the period June 1, 1976 to May 31, 1977. The project is entitled "Use of Unicellular Algae for Evaluation of Potential Aquatic Contaminants." Research was conducted by the Water Resources Laboratory, School of Engineering, University of California, Irvine. The investigation was designed to expand the knowledge of toxic and biostimulatory responses of unicellular algae to hydrazine propellant and to aid Air Force personnel in assessing the environmental impact of compounds which may be released into the aquatic environment.

Contract monitor was Lt. Colonel Roger C. Inman, Chief, Environmental Quality Branch of Toxic Hazards Division, AMRL, Wright Patterson Air Force Base, Dayton, Ohio. Principal investigators were Jan Scherfig, Civil and Environmental Engineering and Peter S. Dixon, Department of Ecology and Evolutionary Biology, University of California, Irvine. Project coordinators were Mrs. Carol A. Justice and Mr. Richard Appleman.

The authors gratefully acknowledge the assistance of Miss Mahin Talebi, Mr. Gregory Goodyear, and Mr. Gregory Roche for their efforts in the overall conduct of the study.

INTRODUCTION

Increasing emphasis on the preservation of environmental quality has made it necessary to determine the effects of substances released into the environment. Hydrazine propellants are used as rocket fuels for current space launch vehicles and are proposed for use in future space systems. Their use presents the possibility of spillage into the aquatic environment and the effects of these compounds in various types of aquatic systems must therefore be determined.

Algal bioassays provide a firm basis for assessing the impact of possible aquatic contaminants on algae over a wide range of nutrient and salinity levels. Algae are particularly significant as major primary producers in all aquatic food chains.

OBJECTIVES

Research objectives for the past year have been directed toward the establishment of dose/concentration responses of unicellular green algae to hydrazine propellants and the determination of compound stability in various aquatic environments as a function of time. Compounds studied include hydrazine, unsymmetrical dimethylhydrazine (UDMH), monomethylhydrazine (MMH), and symmetrical dimethylhydrazine (SDMH). Dose responses have been determined for two algal species using the Standard Algal Bioassay procedure (American Public Health Association, 1975; U.S.E.P.A., 1971). Both fresh water and marine bioassays have been conducted to simulate a range of aquatic ecosystems, such as oligotrophic lakes, eutrophic lakes, lakes of intermediate trophic status, estuaries and the open sea. The overall goals have been to provide information for environmental impact statements and determine threshold limits under which the Air Force can operate within the National Environmental Policy Act. The specific objectives have been:

1. Determining 'no effect' or safe concentration (SC) for the compounds under the various test conditions.
2. Determining EC_{50} for the compounds under various test conditions. The EC_{50} is defined as that concentration which causes a fifty percent decrease in growth at a specified time.
3. Assessing compound stability by studying decomposition rates in dilute solution under various test conditions.
4. Evaluating compound stability by GC Mass-Spectroscopy.

MATERIALS AND METHODS

ALGAL BIOASSAYS

Algal bioassays were conducted in accordance with Standard Methods (American Public Health Association, 1975) and the Algal Assay Procedure: Bottle Test (U.S.E.P.A., 1971) in order to determine the safe concentration (SC) and effective concentration which reduced algal growth by fifty percent (EC_{50}). Compounds tested included hydrazine, unsymmetrical dimethylhydrazine (UDMH) and monomethylhydrazine (MMH).

Algal bioassays were conducted in two steps: (1) a broad screening series and (2) a fine evaluation analysis. First a preliminary series of replicate flasks containing the algal growth medium was dosed with a broad range of concentrations (e.g. from 0.001 to 10 ppm) of the test compound of interest. Flasks were seeded with the appropriate test organism and algal growth (both total cell number and total algal volume) was monitored until at least the control flasks without test compound reached the maximum biomass. The maximum biomass or maximum standing crop is defined as having been achieved when the biomass increase is 5% or less per day. In this way it was possible to determine the approximate concentration where the SC and EC_{50} would be expected to occur. Then another series of flasks containing growth medium was dosed with this narrow range of concentrations of the test compound. All flasks were seeded to an initial concentration of 1×10^6 cells/ℓ with the appropriate algal species. Algal growth was monitored as described above and the SC and EC_{50} concentrations were determined. The Standard Algal Assay Medium (SAAM) was the growth medium for fresh water bioassays and artificial sea water (ASW) was the medium for marine algal assays.

SAFE CONCENTRATIONS (SC)

The 'no effect' or safe concentrations (SC) were determined by using a t-test to compare mean growth in the control flasks with mean growth for each concentration of test compound. The SC is the highest concentration of test compound which causes no statistically significant difference in growth (at the 95% confidence level) when compared with the control flasks without test compound added.

EFFECTIVE CONCENTRATIONS (EC_{50})

Effective concentrations were determined graphically by plotting percent algal growth as a function of the initial concentration of test compound. The EC_{50} is that concentration which causes a 50% reduction in growth when compared with the controls without chemical added. Total cell number was used as the algal growth index; the days elapsing after inoculation varied from compound to compound.

RESULTS AND DISCUSSION

The details of all results have been presented in the monthly reports. The following represents a summary of the information obtained and a discussion of its interpretation.

HYDRAZINE BIOASSAYS

Ten Percent SAAM

The 10 percent SAAM medium is equivalent in nutrient status to freshwater

of oligotrophic conditions.

Five replicate flasks were dosed with concentrations of 0.05, 0.50, 5.0 and 10.0 µℓ of hydrazine per liter of 10% SAAM. These flasks and five controls without hydrazine were seeded with Selenastrum capricornutum to an initial concentration of 1 x 10^6 cells per liter. Algal growth and hydrazine concentrations were monitored daily for the first seven days and then once or twice per week until the 31st day. The results are shown in Table 1.

The analysis of growth shows that a concentration of 0.05 µℓ of hydrazine per liter in 10% SAAM caused a statistically significant reduction in algal growth until day 7 but by day 14, the difference was not statistically significant. Safe concentrations (SC) and effective concentrations (EC_{50}) for hydrazine in 10% SAAM are shown in Table 1. Percent algal growth as a function of initial hydrazine concentration is presented in Figure 1.

TABLE 1

SC AND EC_{50} FOR HYDRAZINE IN 10% SAAM

Day	SC µℓ/ℓ		EC_{50} µℓ/ℓ	
	number	volume	number	volume
4	0.001	0.001	0.02	0.03
7	0.002	0.002	0.02	0.03
14	0.003	0.002	0.07	0.08

Thirty-Three Percent SAAM

This medium is equivalent to conditions found in freshwaters of intermediate nutrient status.

Algal bioassays were conducted utilizing five replicate flasks with 0, 0.01, 0.05, 0.10 and 1.10 µℓ of hydrazine per liter of 33% SAAM. Flasks were seeded with Selenastrum capricornutum and algal growth monitored for 8 days. The results are presented in Table 2 and Figure 2.

Analysis of maximum biomass data indicated that there is no statistically significant difference in cell numbers or volumes between the control group of flasks without hydrazine and those which had an initial concentration of approximately 0.01 µℓ/ℓ. The group of flasks which received an initial hydrazine concentration of 0.05 µℓ/ℓ produced 68% fewer cells and 79% less algal volume as compared with the controls and the 0.01 µℓ/ℓ concentration. These differences are statistically significant at the 99% level. Based on these data, the SC for hydrazine in 33% SAAM under these test conditions is 0.01 µℓ/ℓ. The 8 day EC_{50} based on maximum cell number or volume would be approximately 0.04 µℓ/ℓ. The SC and EC_{50} concentrations for hydrazine in 33% SAAM are presented in Table 2.

TABLE 2

SC AND EC_{50} FOR HYDRAZINE IN 33% SAAM

Day	SC µℓ/ℓ		EC_{50} µℓ/ℓ	
	number	volume	number	volume
3	0.001	0.001	0.013	0.013
8	0.01	0.01	0.037	0.032

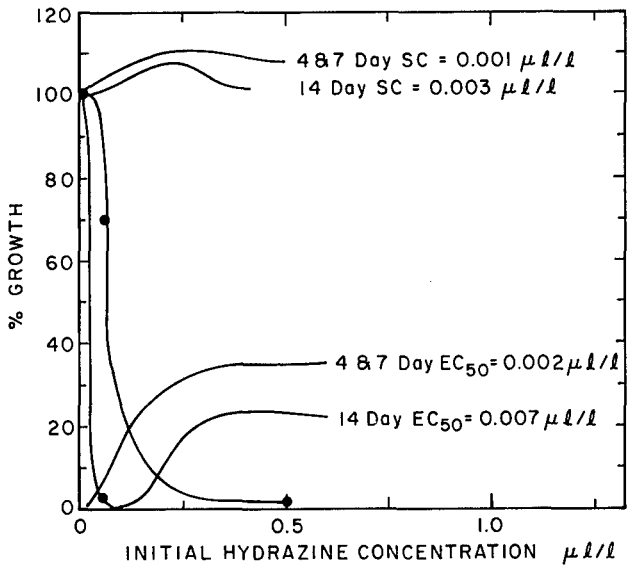

Figure 1. THE EFFECT OF VARYING INITIAL HYDRAZINE CONCENTRATIONS ON CELL NUMBERS OF S. CAPRICORNUTUM IN 10% SAAM

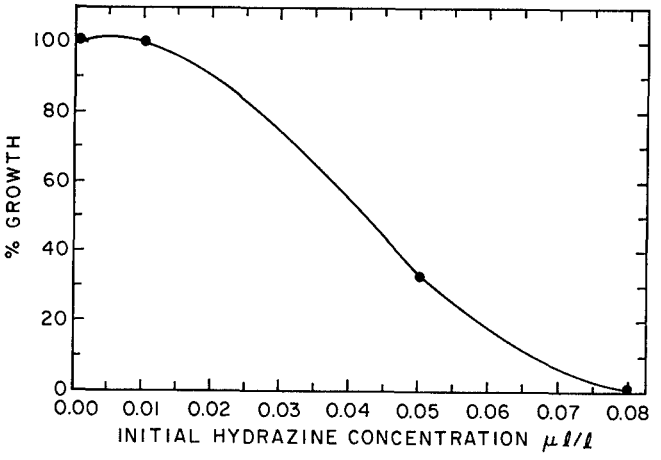

Figure 2. THE EFFECT OF VARYING INITIAL HYDRAZINE CONCENTRATIONS ON CELL NUMBERS OF S. CAPRICORNUTUM AFTER 8 DAYS GROWTH IN 33% SAAM

One Hundred Percent SAAM

This culture medium is equivalent in nutrient status to eutrophic freshwater and is similar in its growth response to high quality secondary sewage effluent.

In the initial experiment, bioassays were established with five replicate flasks containing 0, 8.0, 35, 80, and 137 µℓ of hydrazine per liter of 100% SAAM. Flasks were seeded with Selenastrum capricornutum and algal growth was monitored for a total of 27 days. Algal growth and hydrazine concentration were monitored every few days for 21 days. Control flasks without hydrazine added grew normally but no detectable growth occurred in any experimental flasks. The flasks were reseeded on day 21 but there was still no detectable growth, although the hydrazine concentrations were low enough that algae should have been able to grow. This lack of growth may have been due to (1) the presence of some toxic by-product of hydrazine decomposition, or (2) the possibility that hydrazine may have bound some necessary elements so that they were not available for growth.

The experiment was therefore reestablished, with five replicate flasks for each of the concentrations 0, 0.01, 0.05, 0.10 and 0.15 µℓ of hydrazine per liter of SAAM respectively. Algal growth and hydrazine concentrations were monitored for six days and the results are presented in Table 3 and Figure 3.

Analysis of growth data showed that maximum biomass was reached by the sixth day. Flasks which had an initial hydrazine dose of 0.04 µℓ/ℓ had no detectable concentration (< 0.001 µℓ/ℓ) by the third day. Safe concentrations and effective concentrations are shown in Table 3.

TABLE 3

SC AND EC_{50} FOR HYDRAZINE IN 100% SAAM

Day	SC µℓ/ℓ		EC_{50} µℓ/ℓ	
	number	volume	number	volume
3	0.001	0.001	0.006	0.007
6	0.005	0.005	0.041	0.025

ASW at 35 ppt Salinity, with Ten Percent SAAM Nutrients

This medium is equivalent to seawater of full salinity with the nutrient level equivalent to that of inshore coastal waters.

In a manner similar to that of the preceding experiments, algal bioassays were conducted using five replicate flasks for each of the following concentrations of 0, 0.0005, 0.001, 0.003, 0.005, 0.008 and 0.010 µℓ of hydrazine per liter of medium. Flasks were seeded with Dunaliella tertiolecta and algal growth monitored for eleven days. The results are presented in Table 4.

Analysis of algal growth data shows that 0.0005 µℓ hydrazine per liter of ASW with 10 percent SAAM nutrients caused 33% decrease in algal cell number on day six but on day eleven these flasks were not statistically different from the controls. Safe concentrations and effective concentrations are shown in Table 4.

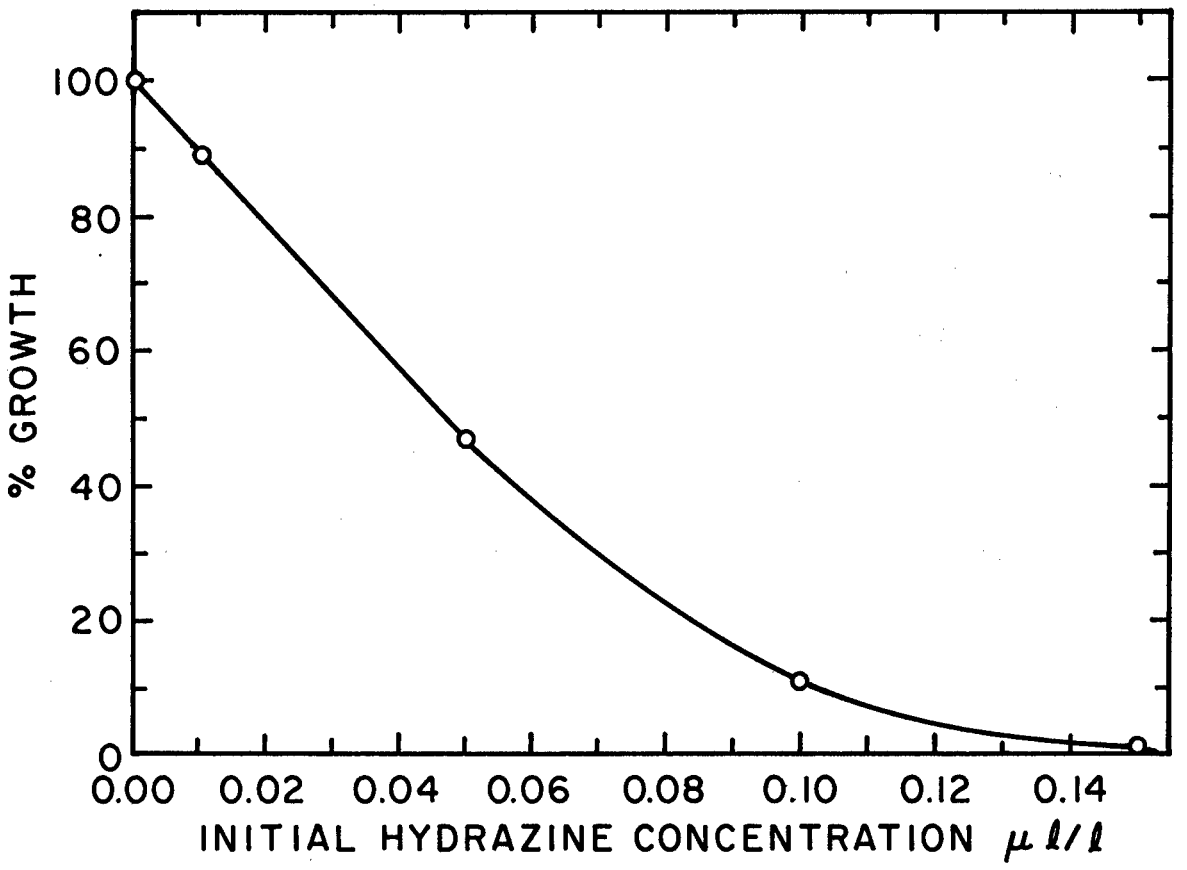

Figure 3. THE EFFECT OF VARYING INITIAL HYDRAZINE CONCENTRATIONS ON CELL NUMBERS OF S. CAPRICORNUTUM AFTER 6 DAYS GROWTH IN 100% SAAM

TABLE 4

SC AND EC_{50} FOR HYDRAZINE IN ASW AT 35 PPT SALINITY AND 10% SAAM NUTRIENTS

Day	SC µℓ/ℓ		EC_{50} µℓ/ℓ	
	number	volume	number	volume
6	< 0.0005	0.0005	0.0008	0.0008
8	0.0005	0.0008	0.0008	0.001
11	0.0005	0.0008	0.002	0.004

ASW at 35 ppt Salinity, with Thirty-three Percent SAAM Nutrients

This growth medium is equivalent to seawater of full salinity but with additional nutrients, such as might occur naturally in estuarine waters.

The initial experiment was set up using five replicate flasks for each of the following concentrations of hydrazine per liter of medium: 0, 0.01, 0.03, 0.05, 0.08, 0.10, and 0.15 µℓ. Flasks were seeded with the marine alga Dunaliella tertiolecta to an initial cell concentration of 1×10^6 cells per liter. Algal growth was monitored for six days. The hydrazine concentrations selected were too high and algal growth did not occur except in the control flasks. The experiment was therefore begun again with five replicate flasks containing 0, 0.001, 0.003, 0.005, 0.008 and 0.01 µℓ of hydrazine per liter of medium, seeded with Dunaliella tertiolecta to an initial cell concentration of 1×10^6 cells per liter. Algal growth was monitored for eight days. Results are shown in Table 5.

Analysis of growth data for the second bioassay shows that a 0.001 µℓ/ℓ concentration caused a statistically significant reduction in algal growth on day six but by day eight there was no significant difference. The safe concentrations and effective concentrations are shown in Table 5.

TABLE 5

SC AND EC_{50} FOR HYDRAZINE IN ASW AT 35 PPT SALINITY AND 33% SAAM NUTRIENTS

Day	SC µℓ/ℓ		EC_{50} µℓ/ℓ	
	number	volume	number	volume
6	< 0.001	< 0.001	0.0011	0.0014
8	0.001	0.001	0.0037	0.0031

UDMH BIOASSAYS

Ten Percent SAAM

As stated previously, this medium is equivalent to oligotrophic freshwater conditions.

In the first investigation, five replicate flasks containing 0, 0.1, 0.5, 1.0, 3.0, 5.0 and 10.0 µℓ of UDMH were seeded with Selenastrum capricornutum. Algal growth was monitored for ten days. Analysis of growth data shows that UDMH concentrations between 0.1 and 6.0 µℓ/ℓ caused a highly significant increase in algal growth (both cell number and volume) when compared to the controls with no UDMH added. Due to the fact that this greatly increased algal growth in the presence of UDMH was not observed in either the 100% and 33% SAAM experiments and that replication among the control flasks was not within the normal limits, this experiment was repeated.

In the second investigation, the initial UDMH concentrations were 0.80, 2.0, 4.0, 6.0, 8.0, and 12 µℓ per liter of 10% SAAM. Five replicate flasks for each concentration and ten replicate flasks without UDMH were seeded with Selenastrum capricornutum to an initial cell concentration of x 10^6 cells/liter. Algal growth was monitored for ten days and results are presented in Table 6.

The addition of 0.8 µℓ of UDMH resulted in 9.5% decrease in algal cell numbers on growth day 6 and this decrease is statistically significant at the 95% level of confidence. However, the next higher concentration of 2.0 µℓ/ℓ did not significantly affect cell number on day 6. The reason for this is not clear but has been observed in other UDMH bioassays and is being investigated. The safe concentrations and effective concentrations are shown in Table 6.

TABLE 6

SC AND EC_{50} FOR UDMH IN 10% SAAM

Day	SC µℓ/ℓ		EC_{50} µℓ/ℓ	
	number	volume	number	volume
6	5.0		< 0.80	2.0
8	5.0		2.0	2.0
11	5.0		2.0	2.0

Thirty-three Percent SAAM

As stated previously, this medium is equivalent to freshwater of intermediate nutrient status.

Algal bioassays were conducted utilizing five replicate flasks containing initial concentrations of 0, 0.1, 0.5, 1.0, 3.0, 5.0 and 10.0 µℓ/ℓ of UDMH per liter of medium. Flasks were seeded with Selenastrum capricornutum and growth was monitored for 10 days. Results are shown in Table 7.

TABLE 7

SC and EC_{50} FOR UDMH IN 33% SAAM

Day	SC µℓ/ℓ		EC_{50} µℓ/ℓ	
	number	volume	number	volume
6	0.5	5.0	4.7	5.4
8	3.01	5.0	6.6	11.7
10	0.5	3.0	10.5	14

One Hundred Percent SAAM

As mentioned previously, this medium is equivalent to freshwater of high nutrient status.

Five replicate flasks containing 0, 0.1, 0.5, 1.0, 3.0, 5.0 and 10 µℓ of UDMH were seeded with Selenastrum capricornutum to an initial cell number of 1 x 10^6 cells/liter. Algal growth was monitored for ten days. Results are presented in Table 8.

Safe concentrations and effective concentrations of UDMH in 100% SAAM are shown in Table 8.

TABLE 8

SC AND EC_{50} FOR UDMH IN 100% SAAM

Day	SC µℓ/ℓ		EC_{50} µℓ/ℓ	
	number	volume	number	volume
6	0.5	0.50	5.3	6.1
8	0.5	5.0	8.0	8.2
10	0.5	1.0	8.0	8.6

ASW at 35 ppt Salinity, with Thirty-three Percent SAAM Nutrients

As mentioned previously, this growth medium is equivalent to seawater of full salinity with additional nutrient content, similar to estuarine water.

Initial concentrations of UDMH were 0, 0.01, 0.05, 0.10, 0.30, 0.50, 1.00 and 3.00 µℓ per liter of medium. Five replicate flasks were prepared for each concentration. All flasks were seeded with Dunaliella tertiolecta and algal growth was monitored for 10 days. Results are presented in Table 9.

Analysis of algal growth data on day ten showed that UDMH concentrations of from 0.01 to 0.5 µℓ/ liter of 33% ASW caused a slight but sometimes statistically significant increase in both cell number and volume. Concentrations of 0.1 µℓ/ℓ of UDMH caused a statistically significant decrease in algal growth. Safe concentrations and effective concentrations are presented in Table 9.

TABLE 9

SC AND EC_{50} FOR UDMH IN ASW AT 35 PPT SALINITY AND 33% SAAM NUTRIENTS

Day	SC µℓ/ℓ		EC_{50} µℓ/ℓ	
	number	volume	number	volume
6	< 0.01	0.1	0.92	0.99
8	0.5	0.3	0.96	1.02
10	0.01	0.1	0.98	1.01

It should be noted that low concentrations of UDMH generally caused an increase in algal growth as compared with the controls without UDMH added and that this increase was often highly significant statistically.

MMH BIOASSAYS

ASW at 35 ppt Salinity, with Thirty-three Percent SAAM Nutrients

As mentioned previously, this growth medium is equivalent to waters found in estuarine conditions.

Initial MMH concentrations were 0, 0.20, 0.40, 0.80, 1.00, 2.00 and 4.00 µℓ per liter of growth medium. Five replicate flasks of each MMH concentration were seeded with Dunaliella teriolecta and algal growth was monitored for ten days. Safe concentrations of MMH are as shown in Table 10.

TABLE 10

SC and EC_{50} FOR MMH IN ASW AT 35 PPT SALINITY AND 33% SAAM NUTRIENTS

Day	SC µℓ/ℓ		EC_{50} µℓ/ℓ	
	number	volume	number	volume
6	0.2	0.4	0.5	0.4
8	0.4	0.4	0.5	0.5
10	0.4	0.4	1.1	1.3

The EC_{50} concentrations described above should be considered the 'worst case' situation. Since the concentration of hydrazine compounds decreases with time in the growth media, the concentration necessary to cause a 50% reduction in growth would increase with time. When the initial concentration is high enough, growth is inhibited permanently, even though the concentration drops to a point which would not be growth inhibiting initially. This indicates that the algal cells are killed or rendered incapable of cell division by a sufficiently high concentration.

Data from bioassays completed to date indicate that of the three hydrazine compounds, UDMH is less harmful under these test conditions and that hydrazine is the most toxic. The safe concentrations and EC_{50} concentrations are lower under marine conditions, even though the compounds are less stable when dissolved in marine media. This may be because the marine test organism is more sensitive to the compounds or that the by-product(s) of decomposition are different under marine conditions and are toxic themselves.

ANALYTICAL DETERMINATIONS

Hydrazine

Hydrazine analyses were performed according to the method described by Reynolds and Thomas (1964). The method has a detection limit of 0.005µℓ per liter and is linear to 0.10 µℓ per liter using 2cm cells. Color development reaches a maximum after 15 minutes and is stable for one hour.

Unsymmetrical dimethlyhydrazine

Analytical determinations for UDMH were carried out according to the method described by Pinkerton *et.al.* (1961). The method has a detection limit of 0.5 µℓ

per liter and is linear to about 30 µℓ per liter. Color development reaches a maximum after about 50 minutes and decreases by approximately 14% for every hour thereafter.

Monomethylhydrazine

Monomethylhydrazine analyses were performed according to the method described by Reynolds and Thomas (1964). The method has a detection limit of 0.05 µℓ per liter and is linear to about 50 µℓ per liter. Maximum color development takes about one hour.

COMPOUND STABILITY IN SOLUTION

Hydrazine

A series of tests were performed to determine the stability of hydrazine in various growth media used for algal bioassays. For the first test, replicate flasks of deionized water, 10% SAAM and 100% SAAM were dosed with a 10 µℓ concentration of hydrazine. Half of the fluids were aerated as in the bioassay procedure and the others were not aerated. Hydrazine concentrations were determined after 1, 7 and 9 days. Results are shown in Table 11.

Hydrazine is more stable in the low medium concentration. There is no statistically significant difference in the initial and final hydrazine concentrations in deionized water.

Results of tests to determine hydrazine stability in 100% SAAM, 100% SAAM macronutrients and 100% SAAM trace metals are shown in Table 12.

TABLE 11

HYDRAZINE STABILITY IN 10% and 100% SAAM

Solution	Percent Change in Concentration		
	22 hrs	7 days	9 days
Deionized water	- 17.0	- 8.3	+ 5.5
Deionized water + aeration	- 7.4	- 7.4	- 0.55
10% SAAM	- 13.3	- 53.9	- 48.9
10% SAAM + aeration	- 11.7	- 22.5	- 18.7
100% SAAM	- 42.1	- 76.2	- 95.3
100% SAAM + aeration	- 62.8	- 95.2	- 98.4

TABLE 12

HYDRAZINE STABILITY AS A FUNCTION OF SOLVENT

Solvent	Hydrazine concentration, µℓ/ℓ Initial	Final	Percent Decrease
Deionized water	9.90 ± 0.71	10.2 ± 0.5	0
100% SAAM	9.63 ± 0.35	0.803 ± 0.109	91.7 ± 0.9
Macronutrients	9.45 ± 0.16	8.85 ± 0.42	6.4 ± 2.9
Trace Metals	9.42 ± 0.40	3.78 ± 0.22	59.9 ± 1.8

The rate of decomposition for hydrazine is dependent upon the ionic matrix in which the compound is dissolved. The highest percentage hydrazine decrease (91%) occurred in 100% SAAM while the trace metal solution caused a mean decrease of 59.9% and the macronutrient solution a mean decrease of 6.4%.

Figures 4,5 and 6 show hydrazine decomposition curves for 10%, 33% and 100% SAAM respectively.

Results of tests to determine the ions in SAAM trace metal solution most responsible for hydrazine decomposition are shown in Table 13 which shows the percent decrease and 95% confidence limit for each solution.

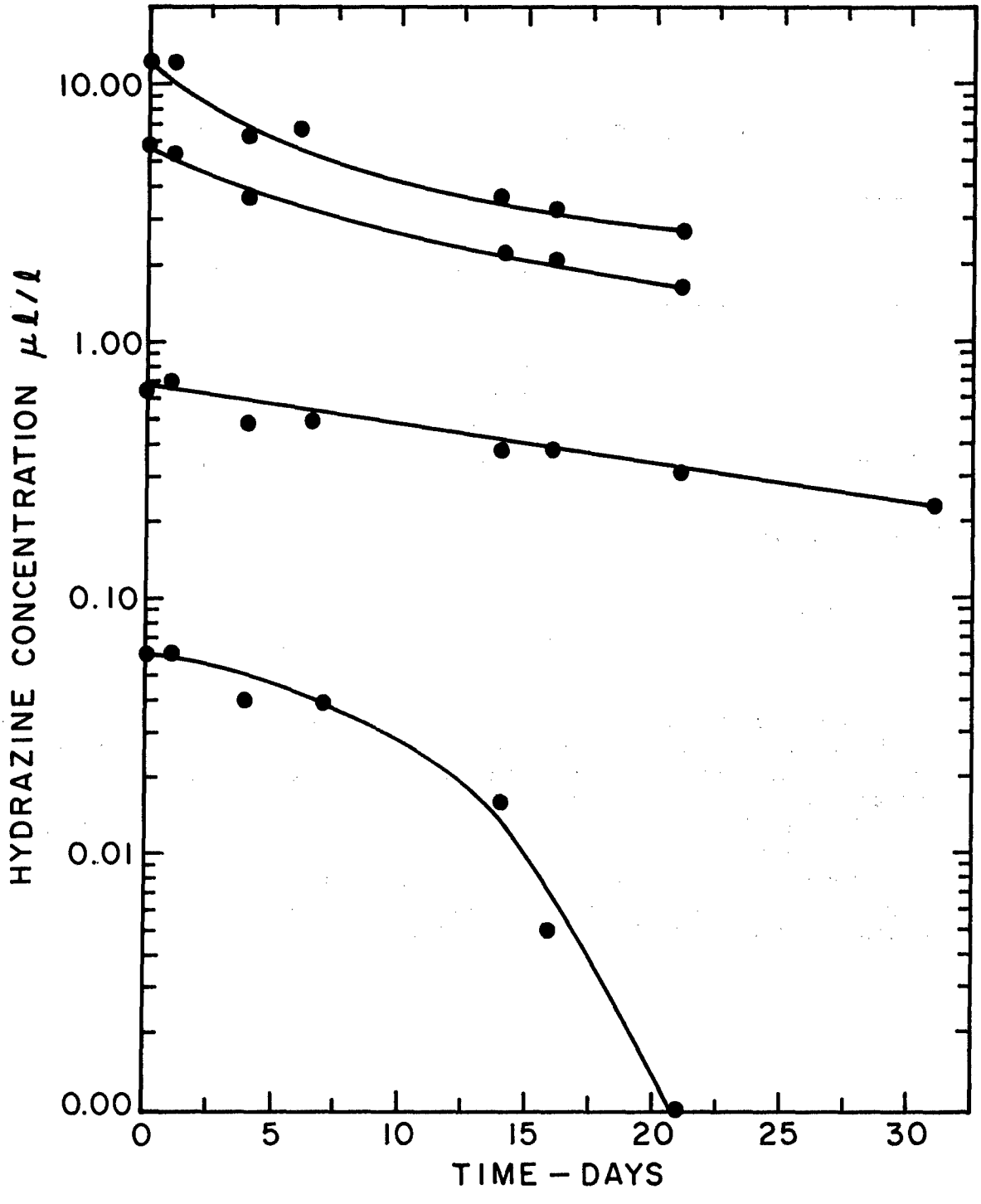

Figure 4. HYDRAZINE DECOMPOSITION IN 10% SAAM AS A FUNCTION OF TIME

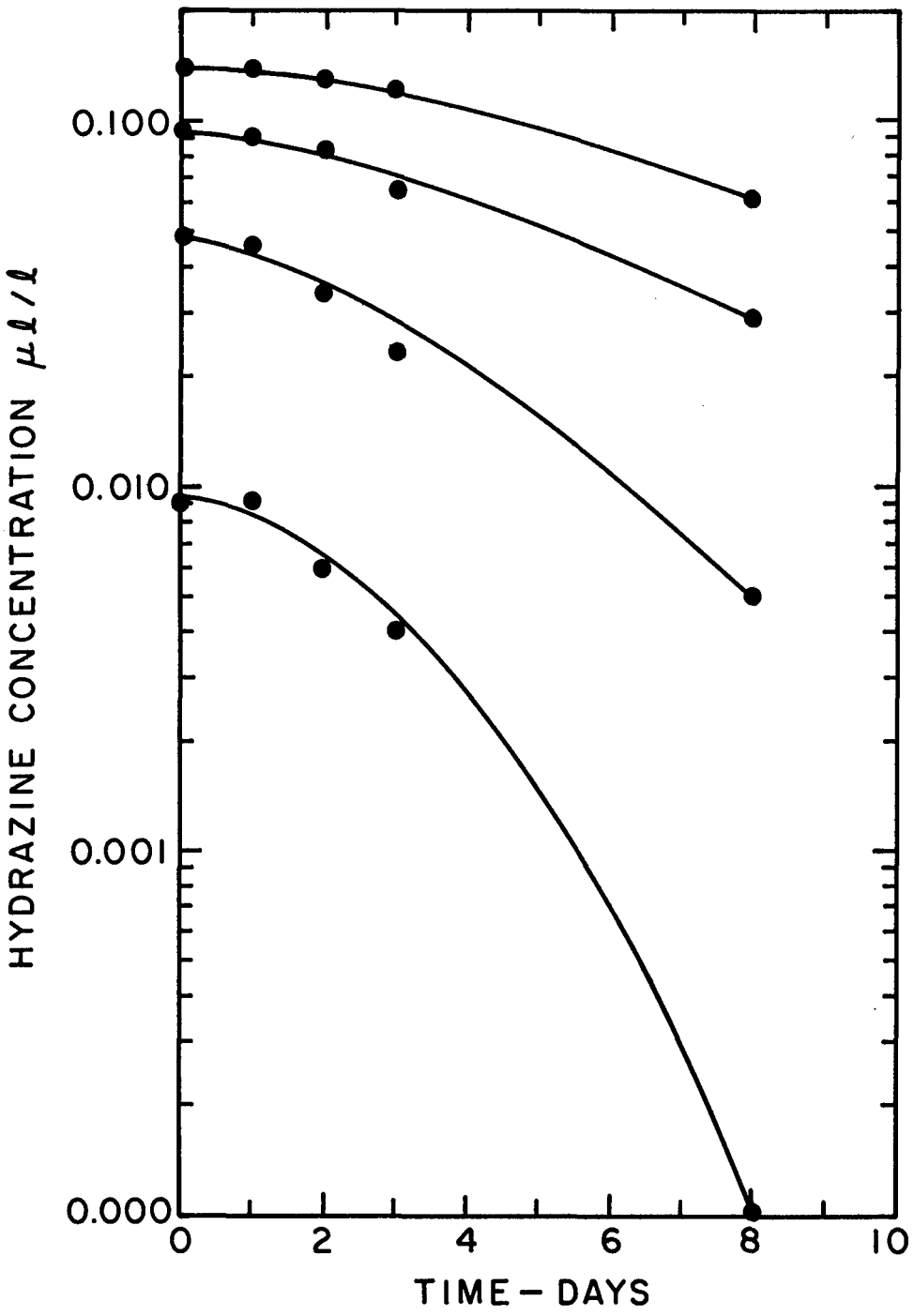

Figure 5. HYDRAZINE DECOMPOSITION IN 33% SAAM AS A FUNCTION OF TIME

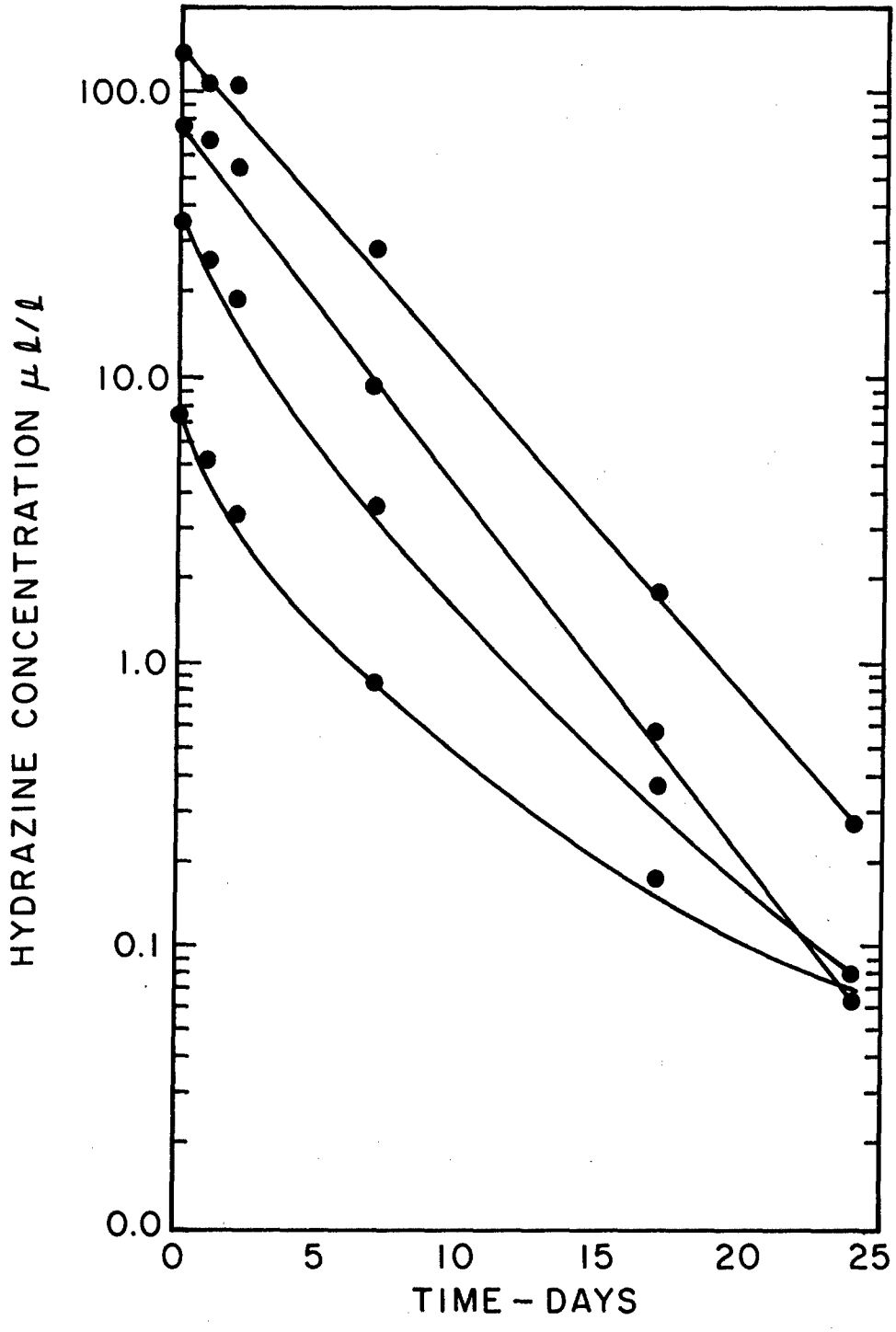

Figure 6. HYDRAZINE DECOMPOSITION IN 100% SAAM AS A FUNCTION OF TIME

TABLE 13

HYDRAZINE STABILITY IN SAAM TRACE METAL SOLUTIONS

Trace Metal Solution	Percent decrease in initial concentrations 4 days	8 days
$FeCl_3 \cdot 6\ H_2O$	28.0	23.2
36 µg Fe/ℓ	± 1.5	± 8.4
$NaMoO_4 \cdot 2\ H_2O$	23.2	13.4
2.9 µg Mo/ℓ	± 0.8	± 1.2
$CuCl_2$	19.4	18.2
0.04 µg Cu/ℓ	± 3.5	--
Na_2EDTA	18.2	17.2
333 µg/ℓ	± 1.2	± 5.7
H_3BO_3	25.0	16.9
32.5 µg B/ℓ	± 10.4	± 3.5
$ZnCl_2$	21.1	21.1
15.7 µh Zn/ℓ	± 0.6	± 13.7
$MnCl_2 \cdot 4\ H_2O$	30.7	62.2
115 µg Mn/ℓ	± 10.8	± 9.8
$CoCl_2 \cdot 6\ H_2O$	20.9	21.2
0.35 µh Co/ℓ	± 1.0	± 8.8
$MgSo_4 \cdot 7\ H_2O$	24.2	15.7
$MgCl_2 \cdot 6\ H_2O$	± 4.4	± 1.4
3mg Mg/ℓ		

Manganese accounted for a 62.2% decrease in eight days but is also the ion of highest concentration. On the basis of a normalized percent decrease or

the basic concentration, copper is by far the most active ion causing a 52.1 percent decrease in hydrazine concentration in eight days. These results agree qualitatively with the published literature as to which ions are active in decomposing hydrazine (Audrieth, L.F. and Ackerson, B., 1951; Audrieth, L.F. and Mohr, P.H., 1951).

Results of tests conducted to determine the effect of Artificial Sea Water (ASW) and copper sulfate on hydrazine stability are presented in Table 14. The percent decrease and 95% confidence limits for each solution are shown.

TABLE 14

THE EFFECT OF VARYING COPPER AND ASW CONCENTRATION ON HYDRAZINE STABILITY

Solvent	Percent decrease in initial concentration Day 4	Day 8	Day 12
$CuSo_4$	23.1	39.3	67.2
10 µg/Cu/ℓ	± 3.7	± 5.1	± 7.2
$CuSo_4$	99.1	99.4	99.5
100 µg/Cu/ℓ	± 9.2	± 0.2	± 0.1
ASW, 10% nutrients	15.0	50.1	> 99.9
@ 35 ppt salinity	± 2.6	± 1.1	--
ASW, 33% nutrients	21.6	63.3	> 99.9
@ 35 ppt salinity	± 8.3	± 25.7	--

Copper Sulfate

A concentration of 100 µg/ℓ Cu will decrease hydrazine by 99.1 percent in four days when the initial hydrazine concentration is 10 µℓ/ℓ . This level of copper is not toxic to most aquatic organisms. In oligotrophic freshwater environments hydrazine will decompose rapidly.

Unsymmetrical Dimethylhydrazine

Results of the tests to determine the stability of 50µℓ/ℓ of UDMH in the algal growth media and deionized water are presented in Table 15. The percent decrease in concentration and 95% confidence limits are shown for a period of nineteen days.

TABLE 15

STABILITY OF UDMH IN ALGAL GROWTH MEDIA

Medium	Percent decrease in initial concentration					
Day	1	3	5	9	13	19
100% SAAM	2.0 ±2.3	9.3 ±2.3	17.9 ±2.8	25.7 ±2.4	38.6 ±3.4	48.5 ±4.1
33% SAAM	0.3 ±4.8	5.6 ±4.8	10.0 ±4.9	12.1 ±4.6	18.6 ±5.5	26.0 ±4.3
10% SAAM	-3.5 ±4.4	1.2 ±5.1	4.1 ±3.8	3.1 ±3.2	8.0 ±4.5	11.7 ±2.8
ASW + 33% nutrients @ 35 ppt salinity	15.1 ±6.2	44.9 ±4.5	59.1 ±5.1	75.7 ±2.6	89.8 ±1.5	98.3 ±1.4
ASW + 10% nutrients @ 35 ppt salinity	12.8 ±7.7	43.6 ±7.0	57.7 ±5.3	78.8 ±2.7	93.3 ±2.2	99.9 --
Deionized water	-1.9 ±2.5	0.1 ±2.2	0.0 ±3.8	-2.3 ±2.9	1.6 ±4.1	9.9 ±3.6

Decomposition is more rapid in marine media and is a function of nutrient concentration in SAAM. Statistically, no decomposition occured in deionized water.

Monomethylhydrazine (MMH)

Results of the tests to determine stability of 50 µℓ/ℓ MMH in algal growth media and deionized water are presented in Table 16. Percent decrease in MMH concentration and 95% confidence limits are shown for a period of twenty-one days.

TABLE 16

STABILITY OF MMH IN ALGAL GROWTH MEDIA

Medium / Day	Percent decrease in initial concentration 1	2	4	6	8	12	21
100% SAAM	23.1 ±0.9	35.2 ±2.6	48.4 ±2.5	56.6 ±1.7	60.4 ±2.5	76.3 ±2.5	89.2 ±2.6
100% SAAM + aeration	22.4 ±1.9	36.0 ±1.8	47.2 ±1.4	56.6 ±1.7	60.9 ±2.7	76.4 ±3.1	89.5 ±2.3
33% SAAM	10.3 ±3.1	21.3 ±2.3	28.0 ±1.7	35.5 ±2.0	36.2 ±1.7	51.5 ±2.3	66.3 ±2.1
10% SAAM	0.2 ±1.7	7.0 ±2.4	10.4 ±1.0	15.7 ±1.2	11.5 ±3.2	29.7 ±3.6	34.3 ±3.1
ASW + 33% nutrients @ 35 ppt Salinity	10.7 ±4.2	45.5 ±7.6	78.6 ±0.8	84.7 ±1.0	87.6 ±0.5	94.7 ±0.5	98.6 ±0.3
ASW + 10% nutrients @ 35 ppt Salinity	19.5 ±3.0	44.6 ±6.6	82.6 ±1.4	89.3 ±1.0	92.5 ±1.0	97.5 ±0.6	99.5 ±0.3
Deionized water	-2.0 ±3.5	-3.4 ±2.4	-2.2 ±3.1	0.1 ±2.3	3.2 ±3.3	4.0 ±2.2	3.0 ±2.5

Decomposition of MMH is more rapid in marine media and is a function of nutrient concentration in SAAM. There was no statistically significant change in MMH concentration in deionized water during this period of time.

Hydrazine, UDMH and MMH are all very unstable in solution and the rate of decomposition is dependent upon the ionic matrix in which the compound is dissolved. All of the compounds decomposed at a more rapid rate when dissolved in marine media than when dissolved in fresh water media. In the fresh water media, the decomposition rate was higher as the nutrient concentration increased:

100% SAAM > 33% SAAM > 10% SAAM

Aeration, as used for the algal bioassays, had no statistically significant effect on the decomposition rates of the compounds.

COMPOUND STABILITY DURING STORAGE

GC/Mass Spectroscopy

Results of the GC/MS analyses for the four hydrazine compounds are shown in Table 17.

TABLE 17

GC/MS ANALYSIS OF HYDRAZINES

Component	Percent by Volume			
	Hydrazine	MMH	UDMH	SDMH
Water	5.49	5.23	3.16	1.10
Methane	--	0.01	0.23	0.19
Methylamine	--	1.88	0.33	0.06
Dimethylamine	--	--	6.7	3.92
Ammonia	5.33	0.33	--	--
Tetrahydrofuran	--	--	2.39	1.79
Aniline	0.21	--	--	--
Hydrazine	88.97	--	--	--
Methylhydrazine	--	92.55	--	--
Dimethylhydrazine	--	--	88.12	92.94

Hydrazine compounds, percent purity (as listed on the label), lot number and manufacturer are as follows:

Hydrazine	Methylhydrazine
95% + %	98%
902/A3D	M5,0001/081447
EASTMAN	Aldrich
1,1 Dimethylhydrazine	Unsymmetrical Dimethylhydrazine
Practical	98%
DX1811/P793	N-24
Matheson Coleman & Bell	ROC/RIC

These results indicate that hydrazine compounds will decompose during storage. Therefore, precautions to prevent loss after opening may have to be instituted, such as displacing the air with nitrogen gas to prevent auto-oxidation in the presence of air and/or moisture absorption. Also, all stocks should be checked prior to conducting toxicity evaluations.

None of the subsidiary components determined within each of the compounds seem to be at high enough levels to account for a significant portion of the toxicities attributed to the major component. Any other components would be less than 0.01 volume percent and therefore would have to be three orders of magnitude more toxic than the major component to account for 10 percent of the effect determined. This rules out most compounds because of the high toxicity of the compounds under testing.

CONCLUSIONS

The EC_{50} concentrations as described should be considered the 'worst case' situation. Since the concentration of hydrazine compounds decreases with time in growth media, the concentration needed to cause a 50% reduction in growth will increase with time. If the initial concentration is sufficiently high, growth is inhibited permanently even though with time the concentration will decrease to a point which would not have been growth-inhibiting initially. This suggests that algal cells are killed or rendered incapable of cell division by a sufficiently high concentration.

Data from bioassays completed indicate that of the three hydrazine compounds tested, UDMH is least harmful under the test conditions employed while hydrazine is by far the most toxic. The safe concentrations and EC_{50} concentrations for all are lower under marine conditions, even though the compounds are less stable in marine media. This difference between freshwater and marine tests may reflect differential sensitivity of the test organisms used. It could also indicate that the by-products of decomposition under marine conditions are themselves toxic.

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